RESEARCH

Forming Core Collections in Barnyard, Kodo, and Little Millets using Morphoagronomic Descriptors

H.D. Upadhyaya,* S.L. Dwivedi, S.K. Singh, S. Singh, M. Vetriventhan, and S. Sharma

ABSTRACT

Millets are hardy crops adapted to marginal lands in hot, drought-prone arid and semiarid environments. Among the small millets, barnyard (*Echinochloa* spp.), kodo (*Paspalum scrobiculatum* L.), and little (*Panicum sumatrense* Roth ex Roem. and Schult.) millets are the most underresearched crops in terms of useful genetic and genomic resources available to breeders for genetic enhancement in these crops. A core collection is an important strategy to enhance use of diverse germplasm with agronomically beneficial traits in applied breeding. The entire germplasm collections of barnyard (736 accessions), kodo (656 accessions), and little (460 accessions) millets at ICRISAT were evaluated for 20 to 21 morphoagronomic traits in two to three rainy seasons at Patancheru, India. Quantitative traits data were subjected to residual (or restricted) maximum likelihood analysis and the best linear unbiased predictors were obtained. Qualitative traits data and standardized data on quantitative traits were used to determine Gower distance matrix, which was subjected to hierarchical cluster analysis following Ward method at R^2 0.75 to form distinct clusters. About 10% or a minimum of one accession from each cluster were selected to form core collections, which consisted of 89 accessions in barnyard, 75 in kodo, and 56 in little millets. Comparisons of means, variances, frequency distribution, diversity indices, and correlations indicated that the variation in the entire collection has been preserved in the core collections, which can be evaluated multilocationally to identify trait-specific diverse germplasm for use in genetic improvement of these crops.

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Abbreviations: CB, culm branching; CR%, coincidence rate; CT, culm thickness; DF, days to 50% flowering; FL, fruit length; FLBL, flag leaf blade length; FLBW, flag leaf blade width; FLSL, flag leaf sheath length; FW, fruit width; G × E, genotype × environment; GH, growth habit; H′, Shannon–Weaver diversity index; IL, inflorescence length; LLR, length of lowest raceme; LRL, longest raceme length; MD%, mean difference percentage; NBT, number of basal tillers; NL, number of leaves; NNPAI, number of nodes on primary axis of inflorescence; NRAT, number of racemes above thumb; NRI, number of racemes per inflorescence; NSIB, number of secondary inflorescence branches; OPAS, overall plant aspects score; PE, panicle exsertion; PH, plant height; PL, peduncle length; PP, plant pigmentation; TL, thumb length; VD%, variance difference percentage; VR% variable rate.

Millets, which are comprised of a number of C_4 small-grained, annual cereal grasses including barnyard millet, kodo millet, and little millet have abundant within-species racial diversity. These species also differ in ploidy levels. The ploidy levels in barnyard millet range from tetraploid to hexaploid to octaploid, while kodo and little millets are tetraploids (de Wet et al., 1983; Wanous, 1990). Barnyard millet has two distinct cultivated species, the Indian barnyard millet [*E. colona* (L.) Link] and Japanese barnyard millet [*E. crus-galli* (L.) Beauv], each with two

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subspecies: *colona* and *frumentacea* in the former and *crus-galli* and *utilis* in the latter. Subspecies *colona* of Indian barnyard millet has no races while subspecies *frumentacea* has four races, stolonifera, intermedia, robusta, and laxa. Subspecies *crus-galli* and *utilis* of Japanese barnyard millet have two races each: crus-galli and macrocarpa in *crus-galli* and utilis and intermedia in *utilis*. Kodo millet is recognized by three races: regularis, irregularis, and variabilis. Little millet has two races, nana and robusta, each with two subraces, laxa and erecta in nana and laxa and compacta in robusta. All these races and subraces can be recognized by variation in panicle morphology (Prasad Rao et al., 1993).

Barnyard millet originated in India and Japan, while kodo and little millets are of Indian origin (de Wet et al., 1983). Barnyard millet is predominantly grown in China, India, Japan, and Korea; kodo millet mainly in India; and little millet in India, Myanmar, Nepal, and Sri Lanka (Prasad Rao et al., 1993). The production and consumption of these millets in India has declined in favor of maize, rice, and wheat (Kumar et al., 2009). The erratic rainfall and drudgery associated with processing have also contributed to a decline in production of these crops (Dwivedi et al., 2012).

The climate model predicts that by the end of 21st century, South Asia will be most adversely affected by climate change and variability. Delayed monsoon season (up to 15 d) accompanied by less summer precipitation and longer breaks between rainy periods are predicted. Similarly, temperature increases of as much as 3 to 4°C have been predicted for India (Ashfaq et al., 2009). Most of the millets, including barnyard, kodo, and little millets, are hardy crops adapted to marginal lands in hot, drought-prone arid and semiarid environments (Dwivedi et al., 2012 and references therein). Unlike other crops, these small millets are less affected by diseases. Smut (*Ustilago panici-frumentacei* Brefeld) in barnyard millet, rust (*Puccinia substriata* Ellis and Barth) and smut (*Sorosporium paspali* and *Ustilago* spp.) in kodo millet, and rust (*Uromyces linearis*) in little millet are the major diseases.

Millets grains are nutritionally equivalent or superior to other cereals. Millets contain high amounts of carbohydrates, proteins, minerals, and vitamins (Mengesha, 1965; Saleh et al., 2012). Thus, millets are most suitable for large-scale utilization in manufacture of food products such as baby foods, snacks foods, and dietary foods. Porridge, chapatti (flat cake or bread made from flour), sprouted grains, dosa (a thin fermented pancake containing blackgram), and popped grains are some of the most common foods prepared from the millets (Dwivedi et al., 2012 and references therein). The beneficial effects of barnyard millet protein on plasma levels of adiponectin, high-density lipoprotein (HDL) cholesterol, glucose, and triglycerides have been documented in obese diabetic mice (Nishizawa et al., 2009). To date, no antinutrients from barnyard and kodo millets have been reported. Kodo millet among all millets has the highest free radical quenching potential, thus possessing good antioxidant property (Taylor and Emmambux, 2008). However, kodua poisoining was reported when kodo millet grains infected with *Aspergillus flavus* or *A. tamarii* were used as food or feed. Both fungi produce cyclopiazonic acid, which causes kodua poisoning (Rao and Husain, 1985), which results in sleepiness, tremors, and giddiness in humans (Bhide, 1962).

Millets in general are an underresearched crop commodity. Recently, barnyard millet has received some attention from the research community in developing genetic and genomic resources, while no systematic crop improvement program exists for kodo or little millets. Plant genetic resources play a critical role in enhancing adaptation and resilience of agricultural production (Sadiki et al., 2007). A reduced subset, which represents diversity of the entire collection of germplasm of a given species, is the way forward to enhance the utilization of germplasm in crop breeding. In this study, the entire germplasm collection of barnyard, kodo, and little millets, preserved in ICRISAT genebank at Patancheru, India, were separately evaluated for taxonomic (species, subspecies, races, subraces) and morphoagronomic traits, which were used to form core collections in these crops.

MATERIALS AND METHODS

Characterizing Barnyard, Kodo, and Little Millets Germplasm for Morphoagronomic Traits

Seven hundred and thirty six accessions of barnyard millet, 656 accessions of kodo millet, and 460 accessions of little millet were characterized for 20 to 21 morphoagronomic traits. Barnyard millet and kodo millet accessions were each evaluated in two rainy seasons: 516 and 220 barnyard millet accessions in 1988 and 2004, respectively, and 286 and 370 kodo millet accessions in 1981 and 2003, respectively. Little millet accessions were evaluated in three rainy seasons: 242, 60, and 158 accessions in 1982, 1985, and 2003, respectively. In each evaluation year, some common entries were added, which were also used to check data quality and in the analysis. The evaluations were done in an alfisols field at Patancheru (78°12′ E, 17°24′ N, and 545 m altitude). Plot size in all the evaluations consisted of one row of 4 m, with an inter- and intrarow spacing of 75 cm and 10 cm, respectively. Diammonium phosphate was applied at 100 kg ha^{-1} as a basal dose and urea at 100 kg ha^{-1} as top dressing 3 wk after planting. The seeds were sown at uniform depth and the crop-specific agronomic practices, including plant protection, were followed to raise the healthy crop.

In barnyard millet, data on 7 qualitative and 14 quantitative traits were recorded following barnyard millet descriptors (IBPGR, 1983a). The qualitative traits were growth habit (GH), plant pigmentation (PP), culm branching (CB), inflorescence compactness, lower raceme shape, lower raceme branching, and overall plant aspects score (OPAS), while the quantitative traits were days to 50% flowering (DF), plant height (PH) (cm), number of basal tillers (NBT), culm thickness (CT) (mm),

number of leaves (NL), flag leaf blade length (FLBL) (mm), flag leaf blade width (FLBW) (mm), flag leaf sheath length (FLSL) (mm), peduncle length (PL) (mm), panicle exsertion (PE) (mm), inflorescence length (IL) (mm), number of racemes per inflorescence (NRI), number of nodes on primary axis of inflorescence (NNPAI), and length of lowest raceme (LLR) (mm).

Kodo millet descriptors included 8 qualitative and 12 quantitative traits, which were recorded following kodo millet descriptors (IBPGR, 1983b). The qualitative traits were GH, CB, leaf erectness, internodal exposure, flag leaf at second primary axis node, spikelet arrangement on the rachis, leaf senescence, and OPAS, while quantitative traits were DF, PH, NBT, NL, FLBL, FLBW, FLSL, IL, sterile primary axis length (SPAL) (mm), number of racemes above thumb (NRAT), thumb length (TL) (mm), and longest raceme length (LRL) (mm).

The 20 little millet descriptors included 6 qualitative and 14 quantitative traits, which were recorded following proso millet (*Panicum miliaceum* L.) and little millet (*P. sumatrense* Roth ex Roem. & Schult) descriptors (IBPGR, 1985). The qualitative traits were GH, PP, CB, inflorescence shape, fruit color, and OPAS, while quantitative traits were DF, PH, NBT, CT, FLBL, FLBW, FLSL, PL, PE, IL, NNPAI, number of secondary inflorescence branches (NSIB), fruit length (FL) (mm), and fruit width (FW) (mm). The DF and qualitative traits in all the three crops were recorded on a plot basis, while quantitative traits were recorded on five representative plants per plot. All the accessions were also taxonomically classified into species–subspecies and races–subraces (Prasad Rao et al., 1993).

Statistical Analysis

The entire data sets of barnyard (21 traits [7 qualitative, 14 quantitative] data on 736 accessions), kodo (20 traits [8 qualitative, 12 quantitative] data on 656 accessions), and little (20 traits [6 qualitative, 14 quantitative] data on 460 accessions) millets (Supplemental Tables S1–S3) were used in the development of core collections. The data on quantitative traits were subjected to residual (or restricted) maximum likelihood (Patterson and Thompson, 1971) analysis in GenStat 14.1 (www.vsni.co.uk; accessed 15 May 2014). Genotypes were considered as random and environment as fixed effect. Best linear unbiased predictors (Schönfeld and Werner, 1986) were estimated and used for analysis. Significance of differences among environments were tested using Wald (1943) statistics. Quantitative data was standardized (Milligan and Cooper, 1988) by range and Gower distance matrix (Gower, 1985) was created for barnyard, kodo, and little millets. The distance matrix was subjected to hierarchical cluster analysis following Ward (1963) method at R^2 (squared multiple correlation) equal to 0.75, using SAS (SAS Institute Inc., 2012) to form distinct clusters. From each cluster, approximately 10% or a minimum of one accession were randomly selected to form the core collection. The core collections were validated on the basis of comparison of frequency distribution of accessions using χ^2 probability for races and qualitative traits. The means and variances were, respectively, tested using Newman–Keuls (Keuls, 1952; Newman, 1939) and Levene (Levene, 1960) tests. The percentage of significant difference between the entire collection and the core collection was calculated for the mean difference percentage (MD%) and the variance difference percentage (VD%). Shannon–Weaver diversity index (H′) (Shannon and Weaver, 1949); the correlation coefficients (Pearson, 1895); the coincidence rate (CR%), and the variable rate (VR%) (Hu et al., 2000) were calculated for quantitative traits to test the representativeness of the core to the entire collection.

Quantitative data was converted into classes using standard deviation (minimum + 1 standard deviation up to maximum) before determining H′, which was computed as $H' = -\sum_{i=1}^{k} p_i \ln p_i$, where k = number of categories and p_i = proportion of accessions in the i^{th} category.

The coincidence rate ($CR\% = \frac{1}{m}\sum_{j=1}^{m}\frac{R_c}{R_e}\times 100$) and the variable rate ($VR\% = \frac{1}{m}\sum_{j=1}^{m}\frac{CV_c}{CV_e}\times 100$) are designed to evaluate the properties of the core collection in terms of the entire collection, where R_c = range of the core collection, R_e = range of the entire collection, CV_c = coefficient of the variation of the core collection, CV_e = coefficient of variation of the entire collection, and m = number of traits. Core collection is considered to be representative of the entire collection if i) no more than 20% of the traits have different means ($P < 0.05$) between the core and the entire collection and ii) the CR% retained by the core collection is no less than 80%.

The correlation coefficient (r) measures the strength and the direction of a linear relationship between two variables and is determined as

$$r = \frac{\sum_{i=1}^{n}(X_i - \overline{X})(Y_i - \overline{Y})}{\sqrt{\sum_{i=1}^{n}(X_i - \overline{X})^2}\sqrt{\sum_{i=1}^{n}(Y_i - \overline{Y})^2}}$$

where x and y are two variables and n is the number of pairs of data. The value of r ranges from −1 to +1, with + and − signs indicating positive linear correlations and negative linear correlations, respectively. A value of r = +1 indicates perfect positive fit, while r = −1 indicates the perfect negative fit. Positive values indicate a relationship between x and y variables such that as values for x increase, values for y also increase, while negative values indicate a relationship between x and y such that as values for x increase, values for y decrease.

RESULTS

In barnyard millet, genotype effects were significant for all traits, while environment effects were significant for most traits (except FLBL, FLBW, FLSL, and IL). Genotype × Environment (G × E) effects were significant for DF, PH, NBT, FLBL, FLBW, FLSL, PL, and IL. In kodo millet, genotype effects were significant for most traits (except NL, FLBL, FLBW, and FLSL), while environment effects were not found for PH and NRAT. The G × E effects were significant for most traits (except IL, SPAL, NRAT, TL, and LRL). In little millet, genotype effects were significant for all traits except for NBT, PL, and PE, while environment effects were not detected for PH, CT, FLBL, and FW. Likewise, G × E effects were not detected for CT, NNPAI, NSIB, FL, and FW (data not given).

Table 1. List of accessions included in core collections of barnyard, kodo, and little millets.

Cluster number	Barnyard millet core collection accessions	Cluster number	Barnyard millet core collection accessions
1	IEc 331, 619, 722, 758	15	IEc 364, 616
2	IEc 178, 179, 183, 198, 208, 217, 229, 240	16	IEc 436, 459, 516, 517, 537
3	IEc 624, 661, 699	17	IEc 731
4	IEc 365, 435, 487	18	IEc 348, 350, 360, 395
5	IEc 404, 423, 448, 498	19	IEc 330, 338
6	IEc 686, 747, 788	20	IEc 449
7	IEc 346, 455, 566	21	IEc 53, 57, 80, 672, 675
8	IEc 452, 471, 511	22	IEc 607, 690
9	IEc 131, 137, 519	23	IEc 67, 321, 353
10	IEc 239, 285, 568, 647, 701,706, 786	24	IEc 301, 316
11	IEc 286, 381, 383	25	IEc 133, 370
12	IEc 100, 269	26	IEc 374, 552, 613, 650, 725
13	IEc 573, 688, 751	27	IEc 648
14	IEc 56, 265, 521, 530	28	IEc 651

(cont'd)

Table 1. Continued.

Cluster number	Kodo millet core collection accessions	Cluster number	Kodo millet core collection accessions
1	IPs 730, 764	28	IPs 292
2	IPs 828	29	IPs 329, 709
3	IPs 695, 919	30	IPs 274, 388
4	IPs 699	31	IPs 622, 645
5	IPs 785	32	IPs 706
6	IPs 181	33	IPs 208
7	IPs 908	34	IPs 627
8	IPs 155, 630	35	IPs 793
9	IPs 245, 319, 358, 614	36	IPs 429, 782
10	IPs 275, 280	37	IPs 599
11	IPs 13, 597	38	IPs 654, 669
12	IPs 777	39	IPs 928
13	IPs 862, 883, 891	40	IPs 279
14	IPs 91, 172	41	IPs 795
15	IPs 236	42	IPs 176
16	IPs 415, 628, 648, 653	43	IPs 741, 870
17	IPs 803	44	IPs 159
18	IPs 670	45	IPs 744
19	IPs 872	46	IPs 368, 585
20	IPs 207	47	IPs 105
21	IPs 694	48	IPs 593
22	IPs 68	49	IPs 606
23	IPs 240	50	IPs 293
24	IPs 318, 344	51	IPs 287
25	IPs 5	52	IPs 4
26	IPs 814	53	IPs 69
27	IPs 254		

Cluster number	Little millet core collection accessions	Cluster number	Little millet core collection accessions
1	IPmr 773	20	IPmr 972
2	IPmr 425	21	IPmr 3
3	IPmr 841, 1039	22	IPmr 847
4	IPmr 917	23	IPmr 864
5	IPmr 733	24	IPmr 741, 758
6	IPmr 961, 967, 1017	25	IPmr 811
7	IPmr 853, 1025	26	IPmr 1057
8	IPmr 854, 878, 1043	27	IPmr 825
9	IPmr 913, 964	28	IPmr 991
10	IPmr 927, 945, 968	29	IPmr 719, 721
11	IPmr 1036	30	IPmr 1075
12	IPmr 851	31	IPmr 10
13	IPmr 771, 774	32	IPmr 828
14	IPmr 708, 718, 1026	33	IPmr 41
15	IPmr 778, 844	34	IPmr 842
16	IPmr 712, 1022	35	IPmr 393
17	IPmr 768, 1008	36	IPmr 386, 843
18	IPmr 867	37	IPmr 894
19	IPmr 62, 414		

A core subset of 89 accessions in barnyard millet was formed, representing three subspecies and eight races. These 89 accessions were selected from 28 distinct clusters. This core subset represented 12% of the 736 barnyard millet germplasm collection maintained at ICRISAT genebank, Patancheru, India (Table 1). The differences between means of the core and the entire collections were not significant for any of the 14 quantitative traits. Likewise, variances between core and entire collections except for PL and LLR were also homogeneous (Table 2). Frequency distribution analysis indicated homogeneity, as detected by χ^2 test, of subspecies and race distribution between the entire collection and the core collection (Table 3). The χ^2 test also revealed the homogeneity in frequency distribution of seven qualitative traits between the core collection and the entire collection (Supplemental Table S4). The H′ is used to measure both allelic richness and allelic evenness. A low H′ indicates an extremely unbalanced frequency of classes for an individual trait and a lack of genetic diversity. The H′ in the core collection was similar to that of the entire collection for 14 quantitative traits (Table 4), which indicates that the diversity of the entire collection was represented in the core collection. The H′ for 7 qualitative traits was also comparable between the core collection and the entire collection (data not presented). The variances and the coefficient of variation in the core collection should be higher than in the entire collection (Hu et al., 2000). The coincidence rate in the core collection for quantitative traits (except for NBT and NNPAI) ranged from 76.8 to 100% (average 82.5%) of the entire collection. The variable rates ranged from 88.1 to 135.7% (average 108.44%) (Table 4). The CR% and VR% for qualitative traits, respectively, were 100% and 97 to 107% (data not presented). The MD% and the VD% between the entire collection and the core collection were 0 and 14.28%, respectively. A proper and adequate sampling has been suggested for the conservation

Table 2. Comparison of means, ranges, and variances for 12 to 14 quantitative traits in the entire and core collections of barnyard, kodo, and little millets.

Trait	Mean		Range		Variance			
	Entire	Core	Entire	Core	Entire	Core	F value	*P*
Barnyard millet								
Days to 50% flowering	48.8a†	49.6a	30.9–77.2	33.2–73.2	36.33	43.37	0.58	0.446
Plant height, cm	93.2a	95.8a	44.5–196.5	57.4–196.5	201.75	283.10	1.43	0.232
Number of basal tillers	7.1a	7.0a	3.9–20.1	4.2–10.9	1.74	1.30	0.36	0.547
Culm thickness, mm	5.5a	5.5a	4.7–7.2	4.9–7.2	0.08	0.09	0.36	0.550
Number of leaves	6.0a	6.1a	5.4–7.2	5.4–6.9	0.05	0.05	0.04	0.850
Flag leaf blade length, mm	205.6a	207.6a	102.8–311.3	127.8–287.9	825.51	883.61	0.10	0.750
Flag leaf blade width, mm	19.6a	19.9a	7.4–32.0	11.3–32.0	13.79	15.18	0.25	0.619
Flag leaf sheath length, mm	88.1a	88.9a	59.4–156.5	66.9–156.5	73.45	114.39	1.90	0.169
Peduncle length, mm	144.6a	144.4a	69.2–277.4	75.4–277.4	389.11	715.02	6.87	0.009
Panicle exsertion, mm	56.5a	55.9a	29.8–80.6	33.9–77.7	46.65	62.99	2.97	0.085
Inflorescence length, mm	155.5a	159.2a	81.0–257.8	102.7–240.8	608.64	617.69	<0.001	0.979
Number of racemes per inflorescence	26.4a	26.7a	21.9–30.4	22.5–29.8	1.77	1.77	<0.001	0.948
Number of nodes on primary axis of inflorescence	10.1a	10.2a	8.7–12.0	9.4–11.3	0.18	0.16	0.66	0.419
Length of lowest raceme, mm	30.0a	30.1a	25.6–38.5	25.6–38.5	1.65	2.68	4.14	0.042
Kodo millet								
Days to 50% flowering	78.7a	77.7a	56.2–117.4	60.0–110.2	52.59	52.88	<0.001	0.980
Plant height, cm	54.6a	54.5a	44.1–69.3	44.3–63.3	11.49	14.84	1.83	0.176
Number of basal tillers	15.3a	15.2a	6.5–30.4	9.2–29.9	11.35	12.44	0.09	0.766
Number of leaves	5.7a	5.7a	5.4–6.7	5.5–6.7	0.01	0.02	3.60	0.064
Flag leaf blade length, mm	191.7a	191.9a	156.1–226.5	156.1–226.5	76.83	102.86	2.28	0.132
Flag leaf blade width, mm	7.2a	7.2a	5.9–8.4	5.9–8.4	0.18	0.18	<0.001	0.985
Flag leaf sheath length, mm	144.8a	144.5a	137.7–151.6	137.7–149.7	4.20	5.56	2.53	0.112
Inflorescence length, mm	64.1a	64.0a	55.4–75.7	57.3–75.7	8.00	10.99	2.40	0.122
Sterile primary axis length, mm	108.2a	108.0a	96.0–123.2	96.0–122.0	14.81	17.64	0.78	0.376
Number of racemes above thumb	3.0a	3.0a	2.7–3.9	2.7–3.9	0.02	0.02	0.28	0.596
Thumb length, mm	56.5a	56.5a	50.3–66.0	52.4–63.7	4.91	5.08	0.01	0.914
Longest raceme length, mm	28.6a	28.6a	25.7–32.4	27.3–32.3	0.57	0.57	<0.001	0.968
Little millet								
Days to 50% flowering	65.0a	67.3a	30.9–139.1	35.0–139.1	108.78	187.05	2.03	0.155
Plant height, cm	112.7a	115.3a	58.3–201.7	60.6–201.7	370.95	477.03	1.01	0.316
Number of basal tillers	13.0a	13.1a	10.4–16.9	11.5–16.2	0.93	1.19	1.06	0.305
Culm thickness, mm	6.0a	6.0a	5.1–7.1	5.3–6.9	0.09	0.09	0.02	0.902
Flag leaf blade length, mm	241.0a	245.5a	175.2–322.9	191.7–322.9	407.83	582.04	2.61	0.107
Flag leaf blade width, mm	32.1a	32.3a	22.6–41.3	23.9–41.3	5.99	7.48	0.47	0.495
Flag leaf sheath length, mm	98.5a	99.1a	81.5–121.3	88.1–114.4	31.18	38.34	0.82	0.365
Peduncle length, mm	159.8a	159.8a	153.3–166.9	154.6–166.7	4.40	5.16	0.41	0.524
Panicle exsertion, mm	21.2a	21.0a	6.9–48.2	9.9–48.2	22.70	33.35	1.74	0.188
Inflorescence length, mm	273.4a	275.1a	198.5–347.6	218.2–330.7	448.75	603.90	1.94	0.164
Number of nodes on primary axis of inflorescence	12.7a	12.7a	11.7–15.0	11.7–14.0	0.13	0.13	<0.001	0.946
Number of secondary inflorescence branches	21.9a	21.9a	17.7–30.0	18.6–30.0	1.12	1.96	1.41	0.235
Fruit length, mm	2.3a	2.3a	2.1–2.4	2.1–2.4	0.003	0.004	3.75	0.053
Fruit width, mm	1.6a	1.6a	1.5–1.8	1.5–1.8	0.003	0.004	2.82	0.094

† Within rows, means followed by the same letter are not significantly different according to Newman-Keul test ($P < 0.05$).

of phenotypic associations arising out of co-adapted gene complexes in the core collection (Ortiz et al., 1998). Phenotypic correlations were conducted between all 14 quantitative traits in the entire and core collections separately. The pattern of correlations was similar in the entire and core collection, demonstrating that associations observed in the entire collection were well preserved in the core collection. Fifty of the 91 correlation coefficients in the core collection were significant ($P = 0.01$) and were in the range from 0.27 to 0.83 (Table 5).

Table 3. Chi square (χ^2) test for the frequency distribution of species, subspecies, races, and subraces in the entire collection and core collection of barnyard, kodo, and little millets.

Species	Subspecies	Race	Subrace	Entire collection	Core collection	df	χ^2	*P*
Barnyard millet								
Echinochloa colona	*frumentacea*			519	61	1	<0.0001	1.000
		stolonifera		43	5	1	0.001	0.981
		intermedia		229	26	1	0.031	0.860
		robusta		202	25	1	0.067	0.796
		laxa		45	5	1	0.016	0.900
		Heterogeneity				3	0.114	0.990
E. crus-galli				217	28	1	0.118	0.731
	crus-galli			134	15	1	0.303	0.582
		crus-galli		114	12	1	0.045	0.831
		macrocarpa		20	3	1	0.259	0.611
		Heterogeneity				1	0.001	0.975
	utilis			83	13	1	0.490	0.484
		utilis		54	8	1	0.025	0.875
		intermedia		29	5	1	0.046	0.830
		Heterogeneity				1	0.419	0.517
Kodo millet								
Paspallum scrobiculatum		regularis		457	48	1	0.376	0.540
		irregularis		82	12	1	0.574	0.449
		variabilis		117	15	1	0.176	0.675
Little millet								
Panicum sumatrense	*sumatrense*	nana		334	39	1	0.068	0.795
			laxa	275	32	1	<0.0001	0.984
			erecta	59	7	1	0.002	0.966
			Heterogeneity			1	0.066	0.798
		robusta		126	17	1	0.180	0.672
			laxa	70	9	1	0.021	0.885
			compacta	56	8	1	0.026	0.872
			Heterogeneity			1	0.133	0.716

In kodo millet, a core subset of 75 accessions was selected from 53 distinct clusters. This core subset represents ~11% of the 656 kodo millet germplasm collection maintained at ICRISAT genebank, Patancheru, India (Table 1). The differences between means of the core collection and the entire collection were not significant for any of the 12 quantitative traits, while the variances were also homogeneous (Table 2). Frequency distribution analysis indicated homogeneity, as detected by χ^2 test, of race distribution between the entire collection and the core collection (Table 3). The χ^2 test also revealed the homogeneity in distribution of eight qualitative traits between the core collection and the entire collection (Supplemental Table S4). The H′ in the core collection was similar to that of the entire collection for 12 quantitative traits (Table 4), which indicates that the diversity of the entire collection was represented in the core collection. The H′ for eight qualitative traits was also comparable between the core collection and the entire collection (data not presented). The coincidence rate in the core collection for quantitative traits ranged from 71.7 to 100% (average 88.09%) of the entire collection. The variable rates ranged from 100.0 to 144.7% (average 111.29%) (Table 4). The CR% and VR% for qualitative traits, respectively, were 100% and 93.2 to 120.7% (data not presented). Both MD% and VD% between the entire collection and the core collection were 0%. The patterns of correlations were similar in the entire and the core collection, demonstrating that associations observed in the entire collection were well preserved in the core collection. Fifteen of the 66 correlation coefficients in the core collection were significant ($P = 0.01$) and were in the range from 0.29 to 0.55 (Table 5).

Little millet core subset consists of 56 accessions, representing two races and four subraces. These 56 accessions were selected from 37 distinct clusters. This core subset represented 12% of the 460 little millet germplasm collection maintained at ICRISAT genebank, Patancheru, India (Table 1). The differences between means of the core collection and the entire collection were not significant for any of the 14 quantitative traits, and the variances (except for FL) were homogeneous (Table 2). Frequency distribution analysis indicated homogeneity, as detected

Table 4. Shannon-Weaver diversity (H′) index, coincidence rate (CR %), and variable rate (VR %) for 12 to 14 quantitative traits in the entire collection and core collection of barnyard, kodo, and little millets.

Trait	H′-index		CR	VR
	Entire	Core	(%)	(%)
Barnyard millet				
Days to 50% flowering	0.630	0.557	86.4	107.5
Plant height, cm	0.611	0.494	91.5	115.2
Number of basal tillers	0.564	0.598	41.5	88.1
Culm thickness, mm	0.626	0.564	92.3	107.3
Number of leaves	0.622	0.581	85.1	102.1
Flag leaf blade length, mm	0.632	0.607	76.8	102.5
Flag leaf blade width, mm	0.624	0.598	84.2	102.9
Flag leaf sheath length, mm	0.621	0.524	92.2	123.7
Peduncle length, mm	0.607	0.559	97.0	135.7
Panicle exsertion, mm	0.632	0.593	86.3	117.4
Inflorescence length, mm	0.632	0.604	78.1	98.4
Number of racemes per inflorescence	0.636	0.609	86.0	99.0
Number of nodes on primary axis of inflorescence	0.621	0.594	57.5	91.4
Length of lowest raceme, mm	0.592	0.571	100.0	127.2
Mean	0.618	0.575	82.50	108.44
SE ±	0.0052	0.0090	4.194	3.673
Kodo millet				
Days to 50% flowering	0.605	0.603	82.0	101.6
Plant height, cm	0.618	0.611	75.4	113.7
Number of basal tillers	0.586	0.537	86.8	105.5
Number of leaves	0.594	0.472	96.9	144.7
Flag leaf blade length, mm	0.626	0.593	100.0	115.6
Flag leaf blade width, mm	0.601	0.537	98.9	100.6
Flag leaf sheath length, mm	0.644	0.618	86.3	115.3
Inflorescence length, mm	0.619	0.581	90.6	117.2
Sterile primary axis length, mm	0.627	0.620	95.6	109.4
Number of racemes above thumb	0.500	0.501	97.5	110.1
Thumb length, mm	0.620	0.573	71.7	101.8
Longest raceme length, mm	0.559	0.494	75.2	100.0
Mean	0.600	0.562	88.09	111.29
SE ±	0.0111	0.0150	2.917	3.545
Little millet				
Days to 50% flowering	0.604	0.458	96.2	126.7
Plant height, cm	0.614	0.580	98.4	110.8
Number of basal tillers	0.626	0.560	72.4	112.4
Culm thickness, mm	0.588	0.612	79.5	98.7
Flag leaf blade length, mm	0.622	0.608	88.8	117.3
Flag leaf blade width, mm	0.549	0.520	93.0	110.9
Flag leaf sheath length, mm	0.617	0.585	66.2	110.2
Peduncle length, mm	0.617	0.566	89.0	108.4
Panicle exsertion, mm	0.588	0.502	92.6	122.2
Inflorescence length, mm	0.641	0.608	75.5	115.3
Number of nodes on primary axis inflorescence	0.578	0.543	68.6	99.5
Number of secondary inflorescence branches	0.563	0.459	92.9	132.4
Fruit length, mm	0.608	0.617	100.0	119.0
Fruit width, mm	0.429	0.433	92.4	108.4
Mean	0.589	0.546	86.10	113.73
SE ±	0.0140	0.0168	3.046	2.512

by χ^2 test, of races and subraces distribution between the entire collection and the core collection (Table 3). The χ^2 test also revealed the homogeneity in distribution of six qualitative traits between the entire collection and the core collection (Supplemental Table S4). The H′ in the core collection was similar to that of the entire collection for 14 quantitative traits (Table 4), which indicates that the diversity of the entire collection was represented in the core collection. The H′ for six qualitative traits was comparable between the core collection and the entire collection (data not presented). The coincidence rate (except for FLSL and NNPAI) in the core collection for quantitative traits ranged from 72.4 to 100.0% (average 86.10%) of the entire collection. The variable rates ranged from 98.7 to 132.4% (average 113.73%) (Table 4). The CR% and VR% for qualitative traits, respectively, were 100% and 93.1 to 103.3% (data not presented). Likewise, the MD% and VD% between the entire collection and the core collection were 0 and 7.14%, respectively. The pattern of correlations was similar in the entire collection and the core collection, demonstrating that associations observed in the entire collection were well preserved in the core collection. Thirty-nine of 91 correlation coefficients in the core collection were significant ($P = 0.01$) and were in the range from 0.32 to 0.67 (Table 5).

DISCUSSION

Natural genetic variation in germplasm collections and its use in applied breeding is one of the most sustainable ways to conserve these valuable genetic resources and simultaneously enhance food and nutritional security in view of the increasing vagaries of climate change in the coming decades. Globally, genebanks have large collections of germplasm of a given species and it is humanly impossible to accurately and effectively evaluate these large collections for morphoagronomic traits, in view of high G × E interactions for quantitative traits. Forming reduced subsets such as core (Frankel, 1984) or mini core (Upadhyaya and Ortiz, 2001) collections, which represent diversity present in the entire collection of a given species in the genebank, is the most sustainable way to accurately and cost-effectively multilocationally evaluate these subsets for identifying agronomically beneficial germplasm for use in crop breeding.

Amongst the millets, barnyard, kodo, and little millets are the most underresearched crop commodity, with practically no useful information available on genetic or genomic resources on these crops. In the present study, we report the formation of core collections, with barnyard millet core containing 89 accessions, kodo millet core 75 accessions, and little millet core 56 accessions (Table 1). Comparison of statistical parameters, including means and variances of quantitative traits, the frequency distribution of qualitative traits, the species and racial diversity, the H′ index, and similarity in correlation coefficients

Table 5. Correlation coefficients in entire and core collections of barnyard, kodo, and little millets. Above the diagonal are correlation coefficients involving core collection accessions; below the diagonal are correlation coefficients involving entire collection accessions.

Trait†	DF	PH	NBT	CT	NL	FLBL	FLBW	FLSL	PL	PE	IL	NRI	NNPAI	LLR
Barnyard millet‡														
DF		0.60	0.02	0.57	0.53	0.40	0.34	0.48	0.08	−0.16	0.55	0.46	0.33	0.29
PH	0.63		0.02	0.47	0.38	0.49	0.31	0.72	0.54	0.24	0.76	0.44	0.37	0.17
NBT	−0.09	−0.25		−0.24	−0.06	−0.23	−0.53	0.14	0.14	0.07	−0.08	−0.27	−0.05	−0.01
CT	0.65	0.66	−0.29		0.65	0.51	0.61	0.27	−0.08	−0.20	0.49	0.45	0.28	0.04
NL	0.62	0.58	−0.20	0.67		0.36	0.39	0.15	−0.21	−0.29	0.41	0.44	0.36	0.04
FLBL	0.52	0.63	−0.30	0.58	0.46		0.65	0.32	0.10	0.05	0.63	0.55	0.36	0.19
FLBW	0.51	0.60	−0.42	0.64	0.50	0.73		0.18	−0.04	−0.07	0.49	0.56	0.28	0.04
FLSL	0.32	0.54	−0.22	0.35	0.22	0.51	0.39		0.60	0.20	0.56	0.19	0.14	0.27
PL	−0.14	0.20	0.08	−0.10	−0.17	0.08	−0.04	0.35		0.83	0.32	0.00	0.02	0.12
PE	−0.27	0.01	0.13	−0.22	−0.26	−0.08	−0.16	−0.03	0.85		0.14	0.01	0.04	0.02
IL	0.48	0.71	−0.30	0.56	0.44	0.67	0.62	0.57	0.11	−0.08		0.62	0.50	0.27
NRI	0.53	0.61	−0.35	0.62	0.56	0.52	0.59	0.30	−0.06	−0.15	0.59		0.67	0.04
NNPAI	0.35	0.42	−0.23	0.35	0.41	0.34	0.37	0.21	0.08	0.03	0.38	0.64		0.17
LLR	0.09	0.16	−0.09	0.09	0.06	0.23	0.18	0.27	0.13	0.03	0.31	0.15	0.16	

Trait	DF	PH	NBT	NL	FLBL	FLBW	FLSL	IL	SPAL	NRAT	TL	LRL
Kodo millet§												
DF		0.42	0.01	0.24	0.23	0.12	0.29	0.40	0.27	0.05	0.43	−0.01
PH	0.49		0.02	0.34	0.29	−0.06	0.47	0.46	0.48	0.16	0.42	0.18
NBT	0.06	−0.01		−0.08	0.17	−0.16	0.09	0.05	0.07	−0.02	−0.10	0.12
NL	0.26	0.36	−0.04		−0.05	−0.04	0.11	0.15	0.14	0.14	0.13	0.08
FLBL	0.11	0.18	0.04	−0.03		−0.29	0.20	0.30	0.27	0.09	0.18	0.00
FLBW	0.13	0.02	0.00	0.05	−0.26		−0.19	−0.15	−0.13	−0.06	−0.05	0.03
FLSL	0.25	0.36	0.02	0.13	0.29	−0.09		0.22	0.33	0.18	0.17	0.06
IL	0.38	0.42	0.01	0.17	0.19	0.01	0.23		0.55	0.02	0.39	0.04
SPAL	0.27	0.45	−0.02	0.16	0.27	−0.04	0.33	0.52		0.08	0.22	0.16
NRAT	−0.07	0.07	−0.01	0.00	0.08	−0.09	0.07	−0.03	0.06		−0.04	0.18
TL	0.39	0.41	0.03	0.18	0.13	0.01	0.30	0.35	0.38	−0.01		−0.02
LRL	0.03	0.12	−0.06	0.08	0.09	0.05	0.08	0.08	0.17	0.09	0.10	

Trait	DF	PH	NBT	CT	FLBL	FLBW	FLSL	PL	PE	IL	NNPAI	NSIB	FL	FW
Little millet¶														
DF		0.47	−0.18	0.45	0.27	0.62	0.29	0.09	−0.17	0.29	0.37	0.33	−0.04	−0.06
PH	0.68		−0.19	0.53	0.44	0.40	0.43	0.13	−0.32	0.63	0.45	0.43	−0.07	0.03
NBT	−0.31	−0.38		−0.20	−0.33	−0.28	−0.07	−0.15	0.22	−0.23	−0.20	−0.19	0.15	0.11
CT	0.72	0.65	−0.28		0.59	0.43	0.48	0.21	−0.32	0.56	0.46	0.65	−0.19	−0.18
FLBL	0.46	0.58	−0.32	0.51		0.39	0.50	0.29	−0.25	0.62	0.60	0.33	−0.03	−0.01
FLBW	0.61	0.53	−0.21	0.60	0.46		0.44	0.55	−0.08	0.39	0.20	0.19	−0.10	−0.07
FLSL	0.24	0.43	−0.16	0.27	0.42	0.34		0.47	−0.40	0.67	0.30	0.26	0.16	0.08
PL	0.10	0.18	−0.05	0.10	0.22	0.43	0.36		0.30	0.43	−0.09	−0.08	0.02	−0.17
PE	−0.15	−0.26	0.14	−0.22	−0.22	−0.03	−0.29	0.56		−0.30	−0.40	−0.38	−0.06	−0.15
IL	0.32	0.64	−0.28	0.37	0.55	0.40	0.57	0.35	−0.23		0.46	0.47	−0.11	−0.02
NNPAI	0.49	0.48	−0.33	0.47	0.40	0.23	0.17	−0.12	−0.21	0.30		0.38	−0.19	0.01
NSIB	0.41	0.44	−0.31	0.51	0.34	0.17	0.19	−0.08	−0.28	0.28	0.49		−0.14	0.05
FL	−0.21	−0.09	0.02	−0.24	−0.07	−0.12	0.03	0.04	−0.01	0.01	−0.17	−0.16		0.48
FW	−0.14	0.00	−0.04	−0.13	−0.04	−0.14	0.05	−0.07	−0.06	0.00	−0.06	0.03	0.51	

† DF, days to 50% flowering; PH, plant height (cm); NBT, number of basal tillers; CT, culm thickness (mm); NL, number of leaves; FLBL, flag leaf blade length (mm); FLBW, flag leaf blade width (mm); FLSL, flag leaf sheath length (mm); PL, peduncle length (mm); PE, panicle exsertion (mm); IL, inflorescence length (mm); NRI, number of racemes per inflorescence; NNPAI, number of nodes on primary axis of inflorescence; LLR, length of lowest raceme (mm); SPAL, sterile primary axis length (mm); NRAT, number of racemes on thumb; TL, thumb length (mm); LRL, longest raceme length (mm); NSIB, number of secondary inflorescence branches; FL, fruit length (mm); FW, fruit width (mm).

‡ Barnyard millet: Coefficients ≥0.27 or ≤−0.27 significant (P = 0.01 at 87 df) in core collection, while those coefficients with ≥0.09 or ≤−0.09 significant (P = 0.01 at 734 df) in entire collection.

§ Kodo millet: Coefficients ≥0.29 or ≤−0.29 significant (P = 0.01 at 73 df) in core collection, while those coefficients with ≥0.10 or ≤−0.10 significant (P = 0.01 at 654 df) in entire collection.

¶ Little millet: Coefficients ≥0.32 or ≤−0.32 significant (P = 0.01 at 54 df) in core collection, while those coefficients with ≥0.12 or ≤−0.12 significant (P = 0.01 at 458 df) in entire collection.

for quantitative traits in the entire collection and the core collection (Tables 2–5), indicate that genetic variation has been adequately preserved in the core collections. Capturing CR% and VR% >80% is ideal. In this study, the core collections of barnyard, kodo, and little millets captured mean CR% of 82.50, 88.09, and 86.10% and mean VR% of 108.44, 111.29, and 113.73%, respectively. These subsets are thus ideal genetic resources for identifying new sources of variation for use in crop improvement. However, small size (56–89 accessions) will probably limit the statistical power of these subsets if used in association genetics for determining marker–trait associations.

The proportion of variance in one trait that can be attributed to its relationship with the other trait is indicated by its coefficient of determination (Snedecor and Cochran, 1980). The correlation coefficients with an absolute value >0.71 or ≤ -0.71 have been suggested to be meaningful (Skinner et al., 1999) as they capture 50% or more of the variation in one trait as predicted by the other. In barnyard millet, 11 correlation coefficients were positive and in the range from 0.60 to 0.83. Of primary interests were DF and PH; FLBL and FLBW; FLSL and PH; PL and FLSL; IL with PH and FLBL; NRI and IL; and NNPAI with NRI. In little millet, six correlation coefficients were in the range from 0.60 to 0.67, with agronomically beneficial associations being IL with PH, FLSL, and FLBL; DF and FLBW; FLBL and NNPAI, or CT and NSIB.

Genes for desirable traits are embedded in biodiversity (Kotschi, 2007). The genetic variation in crops genepool including wild relatives, when systematically characterized, evaluated and documented, and dissected through applied genomic tools provides crop genetic enhancers the agronomically important gene(s) and their allelic forms to develop productive and nutritious crop cultivars. The availability of core collections in these neglected crops provides researchers opportunity to discover new sources of variation for use in genetic improvement of these crops. To date, research at ICRISAT and elsewhere has proven beyond doubt the utility of these representative subsets in identifying agronomically beneficial germplasm for use in crop breeding (Bowman et al., 2001; Brick et al., 2006; Grunwald et al., 2003; Mahuku et al., 2003; Micklas et al., 1999; Tullu et al., 2001; Upadhyaya et al., 2013, 2014). Molecular characterization of these subsets should further provide information on population structure and diversity and allelic richness, which may be used to identify genetically diverse germplasm for use in genomics and applied breeding of these crops. The South Asia region in the 21st century is predicted to be worst affected by global warming. Identification and utilization of genetically diverse and agronomically beneficial germplasm is the most sustainable way to enhance the conservation and utilization of these small millets germplasm in crop breeding. Researchers worldwide can obtain limited seed samples of these accessions from ICRISAT genebank for research purposes through a Standard Materials Transfer Agreement (www.icrisat.org/icrisat-ip-mta.htm, accessed 3 Sept. 2014).

CONCLUSIONS

Reduced subsets (core or mini-core collection) representing diversity of the entire collection of a given species preserved in a genebank are the ideal genetic resources to promote conservation and use of plant genetic resources in crop improvement programs. Core collections reported here in barnyard, kodo, and little millets may be used to discover new sources of variations for enhancing the genetic potential of these small millets.

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